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Title: Method and Composition for Solubilising a Biologically Active Compound with Low Water Solubility

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Country: Europe

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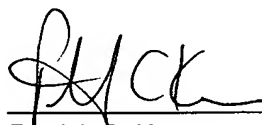
In support of this claim, enclosed is a certified copy(ies) of said foreign application(s). Said prior foreign application(s) is referred to in the oath or declaration. Acknowledgment of receipt of the certified copy(ies) is requested.

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The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

**Patentanmeldung Nr.    Patent application No.    Demande de brevet n°**

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Der Präsident des Europäischen Patentamts;  
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets  
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**I.L.C. HATTEN-HECKMAN**





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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:  
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.  
If no title is shown please refer to the description.  
Si aucun titre n'est indiqué se referer à la description.)

Method and composition for solubilising a biologically active compound with low  
water solubility

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**Method and composition for solubilising a biologically active compound  
with low water solubility**

- 5     The present invention relates to a method and a composition for solubilising a biologically active compound with low water solubility.

In this specification, the following definitions apply:

- 10    “Lipid” refers to membrane lipids which include phospholipids, glycolipids, ceramides, gangliosides and cerebroside. The term as used herein refers to lipids of one single type only as well as to mixtures thereof.

“Lipid suspension” refers to an aqueous dispersion of lipid particles.

“Compounds” are biologically active substances that have a physiological and/or pharmacological effect in a living organism.

- 15    “Container” means an ampoule or vial with rubber stopper and aluminium cap, single or double chamber syringe, infusion bag or bottle made from polymeric materials or glass, suitable for parenteral administration.

- 20    “Low water solubility” means any compound that requires more than 10 parts of water to dissolve 1 part of the compound. It spans the definitions between sparingly soluble (from 10 to 30) to very slightly soluble (from 1000 to 10'000) as defined in USP 24. They are also referred to as lipophilic or hydrophobic compounds.

“Molecular associates” are complexes formed with the compound and the lipids in which the molecules are homogeneously dispersed.

- 25    “Molecular association” between compound and lipid molecules is achieved if not more than 10% of un-associated test material is retained on a 200 nm polycarbonate membrane filter after filtration of at least 1:5 dilution of the lipid mixture containing the test material with distilled water.

“Loading” means incorporating compounds into lipid particles to form molecular associates.

A major problem in delivering biologically active compounds concerns poor aqueous solubility of the compounds. The problem applies in particular to lipophilic compounds that are administered by parenteral or intravenous injection. Because of its low solubility, the compound may precipitate before or after an iv injection or infusion and cause capillary blockage. As a result of precipitation and aggregation, sufficient concentrations of the drug may not be available to bind on to lipoproteins in order to be transported to target receptors and organs. Therefore, up to now it is necessary to solubilise lipophilic compounds to elicit the required therapeutic effects.

In the prior art, ethanol and aqueous solutions of detergents like Cremophor EL® or polysorbate 80 are commonly used to solubilise lipophilic compounds. Alternatively, they may be complexed with hydroxypropyl-beta-cyclodextrins or dissolved in an oil/water emulsion system. However, precipitation of the drug on dilution of the organic solvent is a problem and anaphylaxis following injection with Cremophore EL is not an unknown problem. Oil-in-water emulsions are restricted to compounds with sufficient oil solubility. Furthermore, the compounds may accelerate physical instability of oil-in-water emulsions and some may not be stable to withstand heat sterilisation or storage. Liposomes comprising phospholipid vesicles are sometimes used to deliver poorly water soluble compounds such as amphotericin and doxorubicin. The low toxicity and high tolerability of phospholipids make liposomes an attractive vehicle for delivering lipophilic compounds. However, the wider commercial use of liposomes require complex and expensive manufacturing procedures such as high shear and/or high pressure homogenisation, controlled organic solvent dilution, cross flow filtration to produce aqueous liposomal suspensions containing the drug ( see e.g. Isele, U., Van Hoogevest, P., Hilfiker, R., Capraro, H-G., Schieweck, K. and Leuenberger, H. Large-Scale Production of Liposomes Containing Monomeric Zinc Phthalocyanine by Controlled Dilution of Organic Solvents, J. Pharm. Sci. (1994), 83, 1608-1616.). Even if production problems can be overcome, the majority of drugs and phospholipid are unstable in water and will need to be lyophilised for storage. The problem here is that lyophilised liposomes require expensive synthetic or semi-synthetic lipids with high phase transition temperatures to maintain physical stability on reconstitution, further adding to already high costs.

In numerous prior art references the use of phospholipids to solubilise compounds with low water solubility is described:

Co-pending application PCT/GB98/01803 describes lipid compositions comprising at least one monoacyl lipid eg monoacyl phospholipid and mixtures of mono acyl and diacyl phospholipids that are effective in carrying lipophilic compounds in molecular form. The compositions may be a waxy

solid, a paste-like material or a viscous fluid suitable for filling into hard or soft gelatine capsules. There is no mentioning of an in situ preparation of an injectable drug formulation.

5 The preparation of lipid-drug co-precipitates using diacyl phospholipids to increase the dissolution behaviour of poorly water soluble drug solvates, and the possibility of modifying drug release from such dispersions by incorporating small amounts(<0.05%) of polyvinyl pyrrolidone is described in J. Pharm. Sci. 81, 283-286 (1992). The compositions are prepared essentially by co-precipitation and result in the incorporation of lipid in the crystalline structure of the solvate. The residual solvent trapped in the solvate crystals is given as a possible reason for the improved solubility of the  
10 poorly water soluble compound. An in situ preparation of an injectable drug formulation is not described.

PCT/US86/00637 describes the use of non-esterified fatty acids and monoglycerides together with minor amounts of a monoacyl lipid (lyso phosphatidylcholine) to form solid particles which show  
15 improved oral absorption for various lipophilic compounds. Improved oral absorption is explained to be due to the unique properties in the mixture. An in situ preparation of an injectable drug formulation is not described.

US-A-5,091,188 discloses injectable compositions and methods for rendering insoluble or poorly  
20 soluble powders more stable, by stabilising the external surfaces with one or more layers of phospholipids to prevent the particles of drug from agglomeration during storage. The drug is not in molecular dispersion and an in situ preparation of an injectable formulation is not described.

WO 99/49846 discloses compositions and procedures that yield sub-micron and micron size stable  
25 particles of water-insoluble drugs together with phospholipids, a charged surface modifier and a block copolymer adhered to the surfaces to prevent the particles from particle growth, aggregation or flocculation in suspension. The particles are not in molecular dispersion with the lipid molecules. High shear mixing by means of multiple passes through a micro fluidiser is necessary to reduce the size of the drug particles. There is no mention of an in situ preparation of an injectable drug  
30 formulation.

WO 99/65469 discloses submicron particles of water-insoluble drugs, prepared simultaneously by stabilising microparticulate suspensions of the drug with surface modifier molecules eg a phospho-

lipid, by rapid expansion into an aqueous medium from a compressed solution of the compound and surface modifiers in a liquified gas. The particles of the drug are surface stabilised in the suspension and prevented from agglomerating. The particles are not in molecular dispersion and there is no mention of an in situ preparation of an injectable drug formulation.

5

EP-A-0 795 585 discloses a process for preparing finely divided suspensions of a particulate retinoid or carotinoid in a volatile, organic solvent mixed with aqueous medium in the presence of a physiologically compatible emulsifying agent. An example of the various emulsifying agents used is a hydrolysed lecithin (Emulfluid E) which contains a substantial amount of non polar oils and free fatty acids ie < 45%. The retinoid or carotinoid is not in molecular dispersion. The described compositions are not suitable for parenteral use.

10

EP-B-0 256 090 describes the use of a specific monoacyl lipid, i.e. lyso-phosphatidylethanolamine, alone or in combination with other diacyl phospholipids to solubilise hydrophobic materials inside small unilamellar vesicle (SUV) suspensions. There is no mention of an in situ preparation of an injectable drug formulation.

15

EP-B-0 158 441 relates to pro-liposome compositions based on membrane lipids, to a method of making lipid vesicles by the addition of aqueous fluid to these compositions, and to aqueous dispersions of vesicles. The compositions contain water soluble, or oil-soluble biologically active compounds. They may also contain an organic solvent suitable for injection purposes, such as ethanol. An in situ preparation of an injectable drug formulation is not described.

20

WO97/25977 discloses a process for preparing an oil-in-water fat emulsion composition comprising a cyclosporin, a rapamycin or an ascormycin or a derivative thereof, which comprises the step of admixing to a placebo fat emulsion a concentrate comprising the active, a stabiliser (e.g. phospholipid) and an organic solvent. There is no mention of an in situ preparation of an injectable formulation containing a poorly soluble compound.

25

US-A-5,747,066 and US-A-4,158,707 describe mixed micelles for the aqueous solubilisation of active substances which are only poorly soluble or insoluble in water to obtain a solution with improved storage properties which consist of a phosphatide and a bile salt. There is no mention of an in situ preparation of an injectable drug formulation.

30

US-A-5,192,549 discloses a method of amphipatic drug loading into liposomes by pH gradient, whilst US-A-5,316,771 describes amphipatic drug loading into liposomes by ammonium ion gradient. US-A-5,380,531 also describes a method for an accumulation of amino acids and peptides  
5 into liposomes. These three examples are restricted to loading of preformed liposomes with compounds which are water soluble and possess a basic function which can be protonated.

In US-A-5,616,341, high drug:lipid formulations of liposomal antineoplastic agents are provided. Liposomes may be made by a process that loads the drug by an active mechanism using a trans-  
10 membrane ion gradient, preferably a transmembrane pH gradient. Using this technique, trapping efficiencies approach 100%, and liposomes may be loaded with drug immediately prior to use, eliminating stability problems related to drug retention in the liposomes. Drug:lipid ratios employed are about 3-80 fold higher than for traditional liposome preparations, and the release rate of the drug from the liposomes is reduced. An assay method to determine free antineoplastic agents in  
15 a liposome preparation is also disclosed. The disclosed method is restricted to loading of ionizable antineoplastic agents and the therapeutic use of this approach is clearly intended.

It is apparent that there exists a practical need for an injectable vehicle comprising non-immunogenic and physiological components which can carry effective amounts of poorly water  
20 soluble compounds efficiently. It is therefore an object of the present invention to overcome the difficulties relating to the broader problem of solubilising and maintaining lipophilic compounds in solution in aqueous media and the particular problem in administering lipophilic compounds by injection using a suitable vehicle. A further object of the present invention is to provide an improved method to solubilise hydrophobic compounds as molecular associates in lipid particles, particularly suitable for parenteral use. It is an aim of the invention to provide sterile compositions that  
25 may be prepared in situ, just prior to use, to avoid stability and storage problems. It is a further object of the invention to provide a method that is reproducible, commercially viable and cost effective, practical and can be validated. It is also an object that the components used are safe, readily available and can be rendered sterile. It is yet a further object that the invention may be used in  
30 medical applications, clinical research and pre-clinical screening applications, such as, i.e., in-vitro cell or in-vivo animal efficacy/toxicity studies, and for solubilising compounds in lipid carriers that may be processed further.

The present invention addresses these issues and offers cost effective, simple compositions and a method which avoids production and stability problems. Furthermore, it allows the use of reliable and cost effective phospholipids without the need to use expensive synthetic or semi-synthetic lipids. Unexpectedly, it has been found that a suspension of molecular associates of a poorly soluble compound with membrane lipids may be produced instantaneously by extemporaneous mixing of a  
5 membrane lipid suspension from a first container with a poorly soluble compound from a second container. The poorly soluble compound may be dissolved in a hydrophilic medium or it may be present in a dry powder form. Preferably, the lipid suspension is transparent and the composition is suitable for parenteral, oral, pulmonary and topical administrations to a living organism. The lipid  
10 suspension and the compound are prepared as separate compositions and filled into two separate containers. The lipid suspension is suitable for long term storage and can be manufactured using standard high pressure homogenisers and/or extruders. It may be sterile filtered and in some cases even heat sterilised. The lipophilic compound is dissolved in a hydrophilic medium or presented as a lyophilisate. Shortly before administration the contents of one container is added to the other.  
15 Because of its lipophilicity, the compound partitions spontaneously into the liposomes. Therefore, this method may be referred to as Instantaneous Partitioning Loading (IPL).

Surprisingly it has been found that an aqueous suspension of finely divided lipid particles has a much higher capacity for solubilising lipophilic compounds if the compound is added to already  
20 preformed lipid particles rather than including the components with the lipid during production of the lipid particles. Thus, in accordance with the invention a composition involving two containers for solubilising lipophilic compounds particularly suitable for injection or for other purposes and a method of loading said lipophilic compounds into a preformed suspension of lipid particles are provided. A first container comprises a composition containing at least one membrane lipid, homo-  
25 geneously dispersed in water and optionally, other physiologically acceptable excipients. A second container comprises a lipophilic compound dissolved in a physiologically acceptable solvent or as an amorphous powder. Optionally, other excipients such as stabilisers and preservatives may be included to the compositions in both containers. The contents of the two containers are combined and mixed before use to form molecular associates. Transfer of the lipophilic compound to the lipid  
30 particles via the aqueous phase occurs through partitioning, and the method is instantaneous. The method according to the invention is characterised by a high loading efficiency and practicality. Compared to prior art methods of sequestering lipophilic compounds in liposomes, the loading is straightforward and there is no need for pH alterations or other manipulations to be carried out to remove extraneous material. By the method in accordance with the invention stability and storage  
35 problems are avoided.

- Typically, an aqueous suspension of lipid particles is prepared in bulk. The lipid dispersion comprises between 0.5% w/w to 25% w/w, preferably below 20% w/w, most preferably between 5% w/w to 15% w/w of at least one membrane lipid preferably a phospholipid, suspended in an isotonic, isohydric aqueous medium, optionally containing solvents and surfactants such as bile salts. Any method that gives uniform sub micron particles with a narrow size distribution may be employed. Preferably the aqueous lipid suspension is subjected to high pressure homogenisation or extrusion in a micro-fluidiser to obtain particles below 100nm with a low polydispersity index to produce a transparent or optically clear suspension. The suspension may be produced in volume and transferred into individual unit containers. The suspension may be vesicular, non-vesicular or mixed micellar and may be sterilised by filtration and aseptically filled into individual sterile vials fitted with suitable silicone rubber closures. Alternatively, the vials and contents may be heat sterilised. It is a desired feature that the suspension in the vial is transparent or optically clear.
- 15 The lipophilic compound is prepared either as a solution in a hydrophilic solvent or as an amorphous powder to form molecular associates. For highly lipophilic or unstable compounds, it may be preferable to convert the crystal form to an amorphous form that is more readily soluble. This may be carried out by precipitation and/or lyophilisation with or without stabilisers. The lipophilic compound is held in a second vial either dissolved in a suitable hydrophilic medium or as a lyophilised powder, both containing optionally other excipients.

The content of one of the vials is added to the other as is appropriate and mixed to form molecular associates in situ, just prior to use. The method of loading is rapid and practical and capable of achieving association efficiency of 99% or more. No further processing is required. The suspension is suitable for injection as a bolus dose as such or it may be added as a concentrate to infusion fluids. It may also be used for other purposes e.g. in a nebuliser for inhalation. Furthermore, any unassociated material can be seen clearly in the transparent suspension in the vial and the imperfect composition can be rejected. The small particle size allows the molecular associates to pass a safety filter prior to parenteral administration.

30

The molecular associates formed using the loading method according to the invention remain fully associated and stable for at least 24 hours at ambient conditions, allowing sufficient time for intravenous injection and infusion of highly unstable compounds, such as, for example, cytotoxic com-

- pounds. By comparison most of the prior art methods require loading or solubilisation to be carried out during large scale preparation of the final suspensions. Therefore the final suspension must have a reasonable shelf life to allow storage and transportation. Furthermore, stored prior art suspensions containing lipophilic compounds solubilised in surfactants or trapped in liposomes are likely to fuse and form larger aggregates that could cause embolism. For liposomes, fusion is greatly accelerated when a drug is present. The loading method according to the invention therefore avoids two of the practical problems that limit the commercial use of liposomes as vectors, namely
- 1) cumbersome, costly manufacturing methods,
  - 2) stability issues like maintenance of uniform particle size and avoidance of drug crystals due to liposome fusion and drug aggregation/precipitation.

- It is to be clearly understood that the suspension of lipid particles that are converted to molecular associates with the drug are not limited to liposomes. These may be alternative non vesicular lipid structures which may be totally micellar or mixed micelles, depending on the drug and the application. For intravenous use, vesicular and mixed micellar structures may be preferred. The type of lipid particle obtained depends on the combination of diacyl to monoacyl membrane lipid component and has been described in WO 98/58629 (PCT/GB98/01803) which is hereby incorporated by reference.

#### Lipophilic Compound

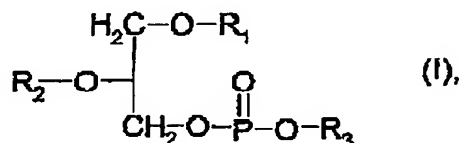
- The invention is particularly suitable for solubilising poorly water soluble compounds that are administered in single doses above about 10mg and have solubilities of less than 10mg/100ml in deionised water at ambient temperature. It is particularly suitable for compounds that have water solubilities of less than 1mg/100ml and lipophilic compounds that bind onto lipoproteins. Typical examples of biologically active lipophilic compounds that have poor water solubility, include hydrophobic immunosuppressants like neutral cyclic peptides eg. cyclosporin A, tacrolimus or a macrolide e.g. a rapamycin.

- It is to be understood further that the lipophilic compound in the second container may include other excipients which are compatible with the compound and facilitate the loading of the lipids. Optionally, the excipients may be bulking agents, membrane lipids, bile salts or salts of fatty acids included as minor components in the composition either in solution, as a co-precipitate or lyophilised powder.

The ratio of drug to lipid is typically between 1:2 to 1:200, preferably 1:5 to 1:100, most preferably 1:5 to 1:50 parts by weight..

### Lipid

- 5 The lipid contains at least one membrane lipid, preferably at least one phospholipid of the formula



wherein

$\text{R}_1$  represents  $\text{C}_{10}$ - $\text{C}_{20}$ acyl;

- 10  $\text{R}_2$  represents hydrogen or  $\text{C}_{10}$ - $\text{C}_{20}$ acyl;

$\text{R}_3$  represents hydrogen, 2-trimethylamino-1-ethyl, 2-amino-1-ethyl,  $\text{C}_1$ - $\text{C}_4$ alkyl,  $\text{C}_1$ - $\text{C}_3$ alkyl substituted by carboxy,  $\text{C}_2$ - $\text{C}_5$ alkyl substituted by carboxy and hydroxy,  $\text{C}_2$ - $\text{C}_3$ alkyl substituted by carboxy and amino, an inositol group or a glyceryl group or a salt of such compound.

- 15 The phospholipid may be neutral or it may be charged. It may be a double chain or a single chain amphipath. Examples of neutral phospholipids with double chains are, phosphatidylcholine (PC), phosphatidylethanolamine (PE) and sphingomyelin. Examples of charged phospholipids are phosphatidic acid (PA), phosphatidyl inositol (PI) and phosphatidylserine (PS) and phosphatidylglycerol (PG). The hydrocarbon chain can either be unsaturated or saturated and can have between 10 to 24, preferably 14 to 18 carbon atoms.

- 20 The single chain lipid is the monoacyl derivative of a neutral or charged phospholipid, but it can also be the monoacyl derivative(s) of glycolipids and sphingolipids. Deacylation may be carried out by phospholipase A2 enzyme hydrolysis or by chemical means. The hydrocarbon chain can either be unsaturated or saturated and can have between 10 to 24, preferably 14 to 18 carbon atoms. The lipids may be derived from natural plant, or animal or microbiological sources, synthesised or partially synthesised, including polyethyleneglycol (PEG) derived monoacyl phospholipids, eg. pegylated monoacyl phosphatidyl ethanolamine.
- 25

- Other membrane lipids, such as glycolipids, ceramides, gangliosides and cerebroside can be used in place of, or in partial replacement of phospholipids. The preferred membrane lipid is phosphatidylcholine (PC). Most preferred diacyl phosphatidylcholine is soy PC, followed by Egg PC, POPC,
- 30

and OOPC. Most preferred monoacyl counterpart is enzyme modified (Phospholipase A2) soy PC, followed by Egg PC, 1-palmitoyl PC, 1-oleoyl PC, 1-stearoyl PC.

- 5 The lipid particles may be comprised entirely of a diacyl lipid or a monoacyl lipid on its own or it may contain mixtures of the monoacyl and diacyl components in any combination obtained by enzyme hydrolysis, depending on the end use.

#### Hydrophilic solvent

- Examples of water miscible, pharmaceutically acceptable solvents are: ethanol, 96% ethanol, absolute glycerol, propylene glycol, ethyl lactate, polyethylene glycol 300, polyethylene glycol 400, 1,3 butandiol, succinic acid diethyl ester, triethyl citrate, dibutyl sebacate, dimethyl acetamide, 10 DMSO, glycerineformal, glycofurol (tetraglycol), isopropanol, lactic acid butyl ester, N-methylpyrrolidone, solketol, propylene carbonate, propylene glycol diacetate, tetrahydrofurfuryl alcohol, diethylene glycol mono ethyl ether, triacetin. Preferably the composition should not contain more than 15% w/w of solvent in the final product after mixing the contents of the two containers, for parenteral or iv use.

- 15 Other pharmaceutically acceptable excipients

- Optionally other pharmaceutically acceptable excipients may be present, either as stabilisers or preservatives. They may be included in the first container holding the lipid suspension or in the second container holding the active compound in solution or as a lyophilised powder. Examples of stabilisers are isotonic and buffer agents e.g. sugars and salts, or anti-oxidants e.g. alpha tocopherol acetate, ascorbyl palmitate. Examples of preservatives are anti-microbials e.g. methyl paraben 20 and butyl paraben. The second vial may also contain excipients which are able to form a cake upon lyophilisation, like polyethylene glycol 3000 and polyethylene glycol 4000, sugars such as mannitol and lactose and saccharose. Furthermore, the second vial may contain other excipients which improve solubility and the loading of the lipids, e.g. mono- and diacyl membrane lipids such as 25 egg PC, soy PC, soy PG, fatty acids and salts thereof, surfactants like polysorbate 80, poloxamer and Cremophor EL.

The following examples are given to illustrate the utility of the invention and not by way of limitation. The invention is not limited to the compounds exemplified or the scale of the production typically shown in the examples.

5    Example 1

10 mg of miconazole and 1mg of POPG are dissolved in 0.25g of ethanol and held in a vial. 2.5g of a 10% phospholipid (98% soya phosphatidylcholine and 2% egg PG) mixture is obtained and hydrated in 10mls distilled water. The lipid dispersion is passed six times through a high pressure homogeniser ( Emulsiflex C5, Avestin) to obtain an optically clear suspension. The lipid particles are smaller than 100nm. The content of the first vial is added to the clear lipid dispersion in the second vial and shaken. The resulting dispersion of molecular associates is clear. Over 90% of the miconazole is transferred to the lipid particles which may be determined by analytical filtration and HPLC analysis.

15    Example 2

10 mg of triclabendazole and 5 mg of sodium oleate are dissolved in 0.5 ml ethanol. This solution is added to 5 ml of deionised water containing 50 mg of lactose. The resultant dispersion is immediately frozen and lyophilised to produce a cake. To this cake 2.5 g of a 10% phospholipid dispersion (98% egg PC) produced by high pressure homogenisation is added. The appearance of the dispersion remains clear after the addition to the cake.

Example 3

1 mg of paclitaxel in a 1 ml container is dissolved in 25 µl of ethanol containing 1 mg of soya PC. To this solution, 0.5 ml of a clear dispersion containing 10% egg PC with a mean diameter of approximately 40 nm is added. The appearance of the dispersion remains a clear transparent dispersion.

Example 4

- To load the lipophilic drug into the lipid dispersion, the contents of vial A should be mixed with the  
5 contents of vial B (or vice versa).

Vial A	Vial B
Aqueous dispersion of lipid particles	Lipophilic drug in water miscible solvent
Aqueous dispersion of lipid particles	Lipophilic drug in dry form obtained from lyophilisation of an organic solvent
Aqueous dispersion of lipid particles	Lipophilic drug in dry form obtained from lyophilisation of an aqueous suspension

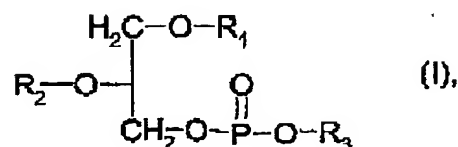
Summary

- 10 The present invention is concerned with compositions for solubilising lipophilic compounds and an improved method of loading poorly soluble compounds into previously formed, clear aqueous suspensions of lipid particles below 1 $\mu$  in diameter, in a vial or other suitable container. It involves mixing an aqueous suspension of lipid particles contained in a first container with a lipophilic compound either in solution or as a lyophilisate in a second container. The compound is transferred  
15 via the aqueous phase to the lipid particles through partitioning, to form molecular associates. The process may be described as Instant Partitioning Loading (IPL). Only minimum agitation is required because of the large surface areas presented by the lipid particles for partitioning. The entire procedure may be carried out instantly in situ, in sealed sterile units just prior to use in the hospital ward or by the bedside. No further processing is required, but further dilution with infusion medi-  
20 um is possible.

Claims:

1. A composition for solubilising a biologically active compound having low water solubility comprising within a first container at least one membrane lipid homogeneously dispersed in an aqueous medium, and, optionally, other physiologically acceptable excipients and further comprising within a second container the biologically active compound having low water solubility, and, optionally, other physiologically acceptable excipients.
2. The composition of claim 1, wherein the membrane lipid is substantially free from non polar lipids.
3. The composition of claims 1 or 2, wherein the membrane lipid is selected from the group consisting of phospholipids, glycolipids, sphingolipids, ceramides, gangliosides and cerebroside.
4. The composition of claim 3, wherein the membrane lipid is monoacyl or diacyl phosphatidylcholine or a mixture thereof, the diacyl phosphatidylcholine preferably being selected from the group comprising soy PC, Egg PC, POPC, and OOPC, and the monoacyl phosphatidylcholine preferably being selected from the group comprising enzyme modified (Phospholipase A2) soy PC, Egg PC, 1-palmitoyl PC, 1-oleoyl PC, and 1-stearoyl PC.

5. The composition according to any one of claims 1 to 4, wherein the membrane lipid is of the formula



5 wherein

$\text{R}_1$  represents  $\text{C}_{10}$ - $\text{C}_{20}$ acyl;

$\text{R}_2$  represents hydrogen or  $\text{C}_{10}$ - $\text{C}_{20}$ acyl; and

10  $\text{R}_3$  represents hydrogen, 2-trimethylamino-1-ethyl, 2-amino-1-ethyl,  $\text{C}_1$ - $\text{C}_4$ alkyl,  $\text{C}_1$ - $\text{C}_3$ alkyl substituted by carboxy,  $\text{C}_2$ - $\text{C}_3$ alkyl substituted by carboxy and hydroxy,  $\text{C}_2$ - $\text{C}_3$ alkyl substituted by carboxy and amino, an inositol group or a glyceryl group or a salt of such compound.

- 15 6. A method of solubilising a biologically active compound having low water solubility which involves mixing the contents of a first container containing at least one membrane lipid homogeneously dispersed in aqueous medium, and, optionally other physiologically acceptable excipients with the contents of a second container containing the biologically active compound with low water solubility, and, optionally, other physiologically acceptable excipients.

- 20 7. The method of claim 6, wherein the contents of the first container includes a lipid dispersion comprising between 0.5% w/w to 25% w/w, preferably below 20% w/w, most preferably between 5% w/w to 15% w/w of at least one membrane lipid preferably a phospholipid, suspended in an isotonic, isohydric aqueous medium, optionally containing solvents and surfactants such as bile salts.

25

8. The method of claims 6 or 7, wherein the aqueous lipid dispersion is subjected to one of high pressure homogenisation and extrusion in a micro fluidiser to obtain particle sizes smaller than 100 nm.

Abstract

There are described a composition for solubilising lipophilic compounds and an improved method  
5 for loading lipophilic biologically active compounds into previously formed, clear aqueous suspen-  
sions of lipid particles having sizes below  $1\mu$  in diameter, in a vial or other suitable container. The  
method involves mixing an aqueous suspension of lipid particles contained in a first container with  
a lipophilic compound either in solution or as an amorphous, preferably lyophilised, powder in a  
second container. The compound is transferred via the aqueous phase to the lipid particles through  
10 partitioning, to form molecular associates. Only minimum agitation is required because of the large  
surface areas presented by the lipid particles for partitioning. The entire procedure may be carried  
out instantly in situ, in sealed sterile units just prior to use in the hospital ward or by the bedside.  
No further processing is required.

9. The method according to one of claims 6 to 8, wherein the contents of the second container is an amorphous powder which is prepared by precipitation and/or lyophilisation from a solution of a lipophilic compound in a solvent.
- 5 10. The method according to one of claims 6 to 9, wherein a composition according to any one of claims 1 to 5 is used to prepare an optically clear formulation comprising the biologically active compound.